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The Effects of Mercury on Developing Larvae of 'Rhithropanopeus harrisii' (Gould). I. Interactions of Temperature, Salinity and Mercury on Larval Development

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16. ABSTRACT

Larvae of the estuarine xanthid crab *Rhithropanopeus harrisii* were reared in the laboratory from hatch through completion of metamorphosis to postlarva in 64 combinations of temperature (20–35 °C), salinity (10–40‰) and mercury (0–20 parts 10^{-9} Hg^{2+}). Multiple linear regression analysis and response surface methodology were used to determine and visually display the individual and interactive effects of the various constant temperature–salinity–mercury combinations on both survival and developmental rates of developing larvae throughout total larval development and for zoeae and megalopae separately, to distinguish any differential sensitivity between the two larval forms. Survival capacity of larvae under a broad range of salinities and temperatures characteristic of temperate estuarine conditions was progressively reduced upon continual exposure to mercury ranging from 5 to 20 parts 10^{-9} Hg^{2+}. Exposure concentrations as low as 5 parts 10^{-9} Hg^{2+} reduced the salinity and temperature plasticity of the normally euryhalinic and eurythermal larvae. Larval survival from hatch to postlarva was affected by both mercury–salinity and mercury–temperature interactions with mercury toxicity increasing under suboptimal temperatures and salinities. Viability of early zoeal stages proved more sensitive to mercury exposure than the final megalopa stage. Exposure to mercury concentrations from 5–20 parts 10^{-9} Hg^{2+} prolonged complete developmental duration by 3 to 4 days with zoeal developmental rates retarded more than megalopal rates. Developmental rates of the megalopa were more reduced by mercury at higher salinities, and both zoeal and megalopal developmental rates were more retarded by mercury at lower temperatures. The reduced plasticity of larvae to estuarine conditions and retarded developmental rates by low mercury concentrations may reduce recruitment into adult benthic populations and alter the distributional patterns of pelagic *R. harrisii* larvae.

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JOURNAL ARTICLE

The Effects of Mercury on Developing Larvae of *Rhithropanopeus harrisii* (Gould)

1. Interactions of Temperature, Salinity and Mercury on Larval Development

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Larvae of the estuarine xanthid crab *Rhithropanopeus harrisii* were reared in the laboratory from hatch through completion of metamorphosis to postlarva in 64 combinations of temperature (20–35 °C), salinity (10–40‰) and mercury (0–20 parts 10^{-9} Hg^{2+}). Multiple linear regression analysis and response surface methodology were used to determine and visually display the individual and interactive effects of the various constant temperature–salinity–mercury combinations on both survival and developmental rates of developing larvae throughout total larval development and for zoeae and megalopae separately, to distinguish any differential sensitivity between the two larval forms. Survival capacity of larvae under a broad range of salinities and temperatures characteristic of temperate estuarine conditions was progressively reduced upon continual exposure to mercury ranging from 5 to 20 parts 10^{-9} Hg^{2+}. Exposure concentrations as low as 5 parts 10^{-9} Hg^{2+} reduced the salinity and temperature plasticity of the normally euryhalinic and eurythermal larvae. Larval survival from hatch to postlarva was affected by both mercury–salinity and mercury–temperature interactions with mercury toxicity increasing under suboptimal temperatures and salinities. Viability of early zoeal stages proved more sensitive to mercury exposure than the final megalopa stage. Exposure to mercury concentrations from 5–20 parts 10^{-9} Hg^{2+} prolonged complete developmental duration by 3 to 4 days with zoeal developmental rates retarded more than megalopal rates. Developmental rates of the megalopa were more reduced by mercury at higher salinities, and both zoeal and megalopal developmental rates were more retarded by mercury at lower temperatures. The reduced plasticity of larvae to estuarine conditions and retarded developmental rates by low mercury concentrations may reduce recruitment into adult benthic populations and alter the distributional patterns of pelagic *R. harrisii* larvae.

Introduction

Temperature and salinity represent the dominant 'ecological master factors' within estuaries, and they may act either singly or in concert to modify both physiological and ecological properties of estuarine species (for reviews see Kinne, 1970, 1971; Alderdice, 1972).

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Recognizing that the benthic adults of estuarine decapods depend upon their pelagic larval stages to replenish and maintain their populations within the estuarine community (Mileikovsky, 1971; Sandifer, 1975; Lockwood, 1976), a number of investigators have examined the effects of these two factors, in combination, on the resistance adaptations and developmental processes of the larval stages of a number of estuarine decapod species (Costlow *et al.*, 1960, 1962, 1966; Lucas, 1972; Sandifer, 1973*a*; Sastry & Vargo, 1977; Young & Hazlett, 1978).

Recent concern over elevated levels of heavy metals in estuarine waters, resulting from localized contamination of rivers draining into estuaries and/or of estuaries proper by domestic and industrial wastes, has prompted numerous research efforts into generally three areas: (1) the occurrence and distribution of these metals in estuarine and marine species collected from various locales, (2) the accumulation, retention and elimination rates of various heavy metals by selected species and (3) the biological effects on estuarine and marine species of exposure to higher than ambient concentrations of heavy metals (for recent reviews, see Reish *et al.*, 1977, 1978; Leland *et al.*, 1978).

In general, comparative studies indicate mercury to be the most toxic of the heavy metals to estuarine organisms (Calabrese *et al.*, 1973; Eisler & Hennekey, 1977). As with other environmental parameters, early developmental stages of estuarine decapods appear less resistant to heavy metal toxicity than the adults (Brown & Ahsannullah, 1971; DeCoursey & Vernberg, 1972; Vernberg *et al.*, 1973*a*, *b*; Ahsannullah & Arnott, 1978). Moreover, recent studies demonstrate that salinity and/or temperature may interact to reduce the resistance of estuarine decapod larvae to heavy metal toxicity and other adverse sublethal responses, including retarded developmental rates of larvae (Vernberg *et al.*, 1973*a*, *b*; Rosenberg & Costlow, 1976; Middaugh & Floyd, 1978; McKenney & Neff, 1979; McKenney & Costlow, 1980).

No data can be found in the literature on the effects of mercury on developing larvae of the ubiquitous estuarine xanthid crab, *Rhithropanopeus harrisii*, a species for which considerable ecological, physiological and biochemical information exists (Costlow *et al.*, 1966; Costlow & Sastry, 1966; Kalber & Costlow, 1966; Whitney, 1969; Frank *et al.*, 1975; Sulkin *et al.*, 1975). The object of the experiments in this study were (1) to determine those concentrations of mercury which are lethal to larvae developing under optimal conditions in the laboratory; (2) to determine for those concentrations of mercury which are not immediately toxic, if sublethal developmental rate reductions occur under optimal conditions for development; (3) to determine if lethal and sublethal criteria of mercury exposure are modified by suboptimal salinity and temperature conditions; and (4) to determine if there exists between the two types of larval forms, the free-swimming zoeal stages and the semi-benthic megalopal stage, any differential sensitivity to mercury under both optimal and suboptimal salinity–temperature conditions.

Materials and methods

The experimental design for this study was a $4\times4\times4$ factorial using salinities of 10, 20, 30 and 40‰, constant temperatures of 20, 25, 30 and 35 °C and mercury concentrations of 0 (unexposed control), 5, 10 and 20 parts 10^{-9} Hg^{2+}. Seawater collected offshore and filtered to the 5-μm levels was diluted with glass-distilled water to obtain the moderate salinities. The extreme salinities were prepared by adjusting the salinities of hypo- and hypersaline solutions separated from partly frozen seawater. The four experimental temperatures were held within $\pm0{\cdot}5$ °C of the desired temperature in constant temperature cabinets maintaining

a 12 : 12 photoperoid. Mercury solutions were prepared daily in filtered seawater of the appropriate salinity from a secondary stock of 1 part 10^{-6} Hg^{2+} in glass-distilled water. The secondary stock was made up at least every third day from a primary stock containing 1·36 g l^{-1} $HgCl_2$ in glass-distilled water. Analyses of mercury solutions by atomic absorption spectrophotometry revealed no significant loss of mercury from either the primary stock nor the secondary stocks during the course of the study. The final mercury solutions, however, did demonstrate a loss of mercury from the initial concentrations dependent on both concentrations and temperature (Table 1). For convenience, the mercury concentrations used throughout this paper represent initial concentrations only.

To obtain larvae for the study, ovigerous females of *Rhithropanopeus harrisii* were collected in brackish waters of Sykes Creek located in an estuary between the Indian and the Banana Rivers near Cocoa Beach, Florida (U.S.A.) and shipped via commercial airlines to the Duke University Marine Laboratory in Beaufort, North Carolina (U.S.A.). The females were individually maintained at 25 °C in large glass culture bowls with a diameter of 19·4 cm containing filtered seawater at 25‰ *S* until the release of zoeae. Upon hatching, larvae were transferred at hourly intervals in graded steps of 5‰ *S* from the original 25‰ *S* to the four experimental salinities of 10, 20, 30 and 40‰. This time period was suggested by Kalber & Costlow (1966) to allow for osmoregulatory equilibrium for normal larvae of *R. harrisii* at these salinities. Larvae were reared throughout development in the four experimental salinities at the four constant temperatures of 20, 25, 30 and 35 °C, and under exposure concentrations of 0, 5, 10 and 20 parts 10^{-9} Hg^{2+}.

Larvae were reared 10 per bowl in glass culture bowls with a diameter of 8·9 cm in an approximate liquid volume of 50 ml. Each replicate consisted of five bowls with a total of 50 larvae per experimental condition. Four replicates were performed at each of the 64 temperature–salinity–mercury. Therefore, responses were determined from a total of 12 800 larvae.

Zoeae were transferred daily by large bore pipettes to clean bowls containing filtered seawater of the same salinity, temperature and mercury level and, at the same time, freshly hatched nauplii of *Artemia salina* were added to each bowl. Daily records were kept on mortality with the criteria for death being cessation of heart beat and the time for development to megalopa was recorded for each zoea. Upon reaching the megalopal stage, the larvae were individually segregated in compartmented polypropylene boxes, 32·5 × 22·7 cm, in an approximate liquid volume of 25 ml. Fresh media were introduced daily into each compartment, at which time *Artemia* nauplii were added. Daily mortality data were collected and developmental time to first crab was determined for each megalopa.

Multiple linear regression analysis was performed on both arcsine transformed percentage survival data and developmental duration data for zoeae, megalopae, and for total development from hatch to first crab using the three-factor second-order polynomial equation:

$$\hat{Y} = b_0x_0+b_1x_1+b_{11}x_1^2+b_2x_2+b_{22}x_2^2+b_3x_3+b_{33}x_3^2 + b_{12}x_1x_2+b_{13}x_1x_3+b_{23}x_2x_3, \quad (1)$$

where $\hat{Y}$ = the estimated arcsine $\sqrt{\text{percentage survival}}$ or developmental duration in days for the appropriate larval form and x_0 = intercept; x_1, x_2 and x_3 = the linear effects of mercury (Hg), salinity (*S*) and temperature (*T*),respectively; x_1^2, x_2^2 and x_3^2 = the quadratic effects of mercury (Hg), salinity (*S*) and temperature (*T*), respectively; x_1x_2, x_1x_3 and x_2x_3 = the linear × linear interaction between mercury and salinity (Hg × *S*), mercury and temperature (Hg × *T*) and salinity and temperature (*S* × *T*), respectively; and b_0, b_1, etc. = the regression coefficients. Separate equations were produced at each level of one factor

TABLE 1. Actual concentrations of mercury experienced by larvae of *R. harrisii* at four temperatures over a 24-h period. The mean value of three replicates in parts 10^{-9} Hg^{2+} and the standard deviation is given

Temperature (°C):	20			25			30			35		
Nominal conc.:	5	10	20	5	10	20	5	10	20	5	10	20
Initial conc.	4·92±0·19	9·77±0·31	20·07±0·40	4·92±0·19	9·77±0·31	20·07±0·40	4·92±0·19	9·77±0·31	20·07±0·40	4·92±0·19	9·77±0·31	20·07±0·40
Conc. after 1 h	4·51±0·11	9·37±0·54	19·00±0·40	4·42±0·20	9·10±0·56	18·73±0·61	3·95±0·09	9·23±0·85	19·00±0·40	4·27±0·25	9·33±0·38	18·73±0·83
Conc. after 4 h	3·56±0·09	8·92±0·25	18·23±0·64	3·18±0·06	8·76±0·10	17·97±0·40	2·99±0·28	8·56±0·28	15·83±0·58	2·98±0·29	8·11±0·38	14·47±1·15
Conc. after 12 h	2·50±0·32	7·00±0·55	16·33±0·38	2·12±0·02	6·94±0·44	14·73±0·76	1·85±0·21	5·15±0·61	12·83±0·20	1·74±0·05	4·57±0·10	10·68±2·10
Conc. after 24 h	1·90±0·17	5·37±0·28	13·47±0·24	1·67±0·26	3·98±0·20	8·53±0·64	1·40±0·30	2·23±0·29	4·98±1·20	1·28±0·09	1·92±0·03	3·10±0·43

(mercury) and employed to generate response surface curves at various levels of the two remaining factors (salinity and temperature) (Box, 1954; Box & Youle, 1955; Myers, 1971; Alderdice, 1972). Canonical reduction of these final equations produced the location and absolute value of the maximum or minimum response at the centre of each surface (Box, 1956).

Results

Zoeal survival

Preliminary studies established that under optimal developmental conditions for the larvae of *Rhithropanopeus harrisii*, i.e. 20‰ *S* and 25 °C (Costlow *et al.*, 1966), all freshly hatched larvae died in less than 24 h when exposed to 40 parts 10^{-9} Hg^{2+}. Differential mortality occurred among zoeae reared under 64 combinations of salinity, temperature and mercury with mortality increasing with increasing mercury concentrations from 5 to 20 parts 10^{-9} Hg^{2+} and dependent on both salinity and temperature conditions (Figure 1).

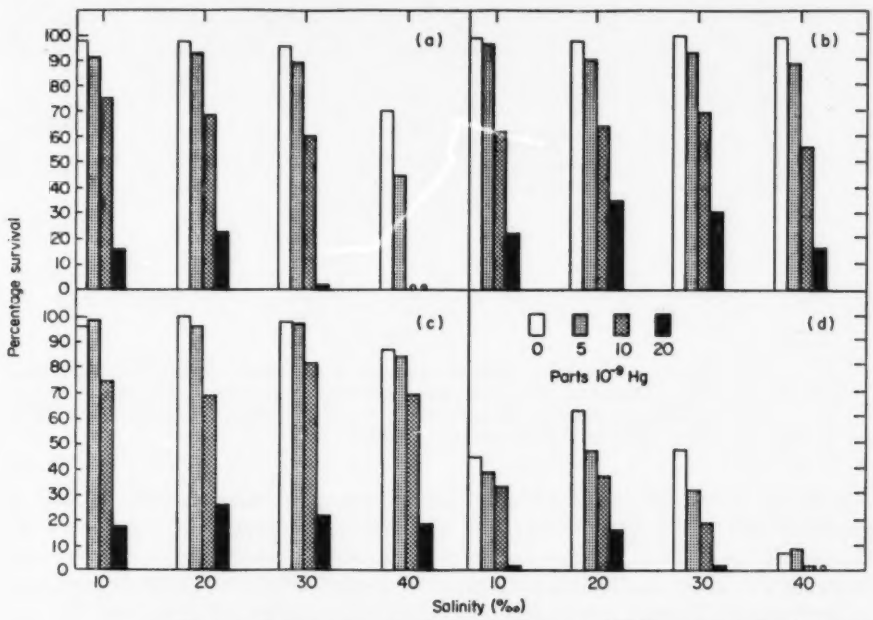

Figure 1. Percentage survival of all zoeal stages of *Rhithropanopeus harrisii* reared from hatch to megalopa in 64 combinations of salinity, temperature and mercury. (a) 20 °C, (b) 25 °C, (c) 30 °C and (d) 35 °C.

Multiple linear regression analysis performed on arcsine transformed percentage survival data produced a second order polynomial expression explaining 83% of the variation:

$$\hat{Y}_1 = -198{\cdot}40 - 5{\cdot}3880\mathrm{Hg} - 0{\cdot}0408\mathrm{Hg}^2 + 0{\cdot}9780S - 0{\cdot}0464S^2 + 23{\cdot}1891T - 0{\cdot}4741T^2 + 0{\cdot}0188\mathrm{Hg}\times S + 0{\cdot}1072\mathrm{Hg}\times T + 0{\cdot}0224S\times T, \quad (2)$$

where $\hat{Y}$ = the estimated arcsine $\sqrt{\text{percentage survival}}$ of the zoeae and the other terms follow the general model [equation (1)] described earlier.

Contour plots of equation (2) depict the pronounced reduction in survival capacity of zoeae reared under various salinity and temperature conditions with increasing mercury exposure (Figure 2). The absolute value of maximum survival decreased from 98% under optimal salinity and temperature conditions for larvae unexposed to mercury to a maximum of 36% survival for larvae exposed to 20 parts 10^{-9} Hg^{2+}. Ninety-five per cent of zoeae not exposed to mercury survived to megalopa under a wide range of salinities (0–37‰) and temperatures (20–32 °C), while only 25% of the zoeae survived to megalopa when exposed to 20 parts 10^{-9} Hg^{2+} under a more restricted range of salinities (9–32‰) and temperatures (24–30 °C).

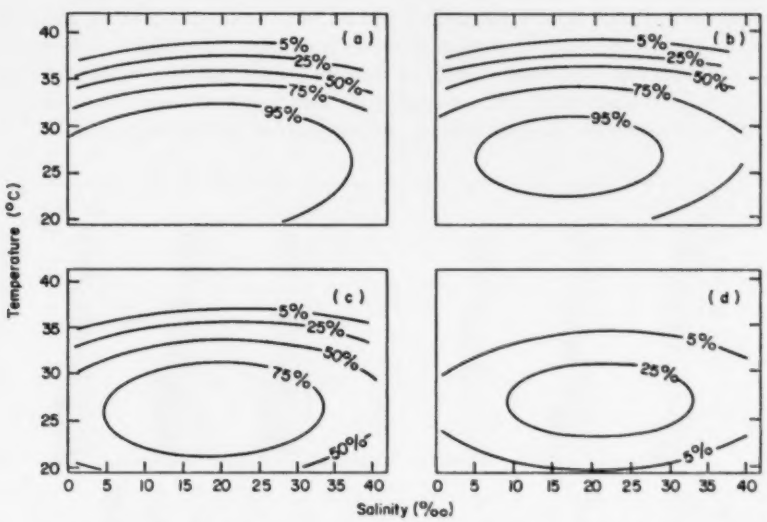

Figure 2. Estimation of percentage survival of all zoeal stages of *Rhithropanopeus harrisii* based on fitted response to observed survival under 64 combinations of salinity, temperature and mercury. (a) 0 parts 10^{-9} Hg^{2+}, (b) 5 parts 10^{-9} Hg^{2+}, (c) 10 parts 10^{-9} Hg^{2+} and (d) 20 parts 10^{-9} Hg^{2+}.

As shown by the contours in Figure 2, mercury exposure decreased zoeal plasticity to salinity at both the upper and lower ends of the salinity tolerance range of the species. However, a significant ($P = 0{\cdot}0007$) mercury–temperature interaction exists, whereby with increasing concentrations of mercury the upper temperature tolerance level for the maximum survival contour of zoeae is consistently near 30 °C, while the lower temperature tolerance level is shifted upward from less than 20 to 24 °C. This mercury–temperature interaction is further evident in the upward shift of the optimal temperature for zoeal survival from 24·8 °C for larvae not exposed to mercury to 27·2 °C for larvae exposed to 20 parts 10^{-9} Hg^{2+}.

Megalopal survival

As with zoeae, survival of megalopa decreased with exposure to increasing concentrations of mercury from 5 to 20 parts 10^{-9} Hg^{2+}, with this response to mercury varying in relation to salinity and temperature conditions (Figure 3). Resistance to mercury by megalopae, as with zoeae, improved under intermediate temperatures between 25 and 30 °C. At the extreme temperatures of 20 and 35 °C, the tolerance of megalopae to mercury decreased at salinities

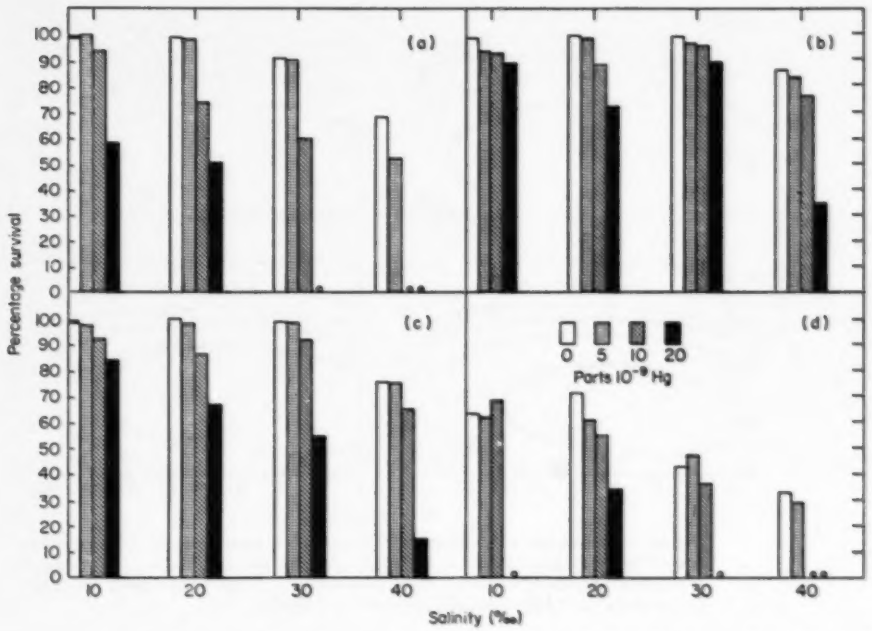

Figure 3. Percentage survival of megalopae of *Rhithropanopeus harrisii* reared under 64 combinations of salinity, temperature and mercury. (a) 20 °C, (b) 25 °C, (c) 30 °C and (d) 35 °C.

above 20‰. In general, the developing megalopae, however, appeared more resistant to mercury exposure than did zoeae.

The polynomial equation generated by regression analysis of the megalopal survival data, explaining 71% of the variation, is

$$\hat{Y} = -184{\cdot}15 - 2{\cdot}8817\mathrm{Hg} - 0{\cdot}0258\mathrm{Hg}^2 - 0{\cdot}0469S - 0{\cdot}0387S^2 + 22{\cdot}5325T - 0{\cdot}4641T^2 - 0{\cdot}0004\mathrm{Hg}\times S + 0{\cdot}0872\mathrm{Hg}\times T + 0{\cdot}0439S\times T, \quad (3)$$

where $\hat{Y}_2$ = the estimated arcsine $\sqrt{\text{percentage survival}}$ of megalopae and the other terms follow the general model [equation (1)] described earlier.

Response surface curves developed from equation (3) are shown in Figure 4. Rotation of the horizontal axis of the contours, regardless of mercury condition, demonstrates the significant ($P = 0{\cdot}018$) salinity–temperature interaction, indicating that similar survival rates of megalopa with increasing temperatures is accompanied by a slight increase in salinity. The absolute maximum survival capacity of megalopa reared under a range of salinities and temperatures is reduced upon exposure to 20 parts 10^{-9} Hg^{2+} from 99 to 92%, a significantly smaller reduction in resistance capacity as compared to the response of zoeae.

Slight, but insignificant, changes in the salinity plasticity of megalopae are associated with mercury exposure (Figure 4). Conversely, as with zoeae, mercury interacts significantly ($P = 0{\cdot}002$) with temperature to produce higher mortality of megalopae at the lower level of temperature tolerance range than at the upper level. Similarly, the optimal temperature for survival of megalopae shifts upward from 24·9 to 26·8 °C with exposure to increasing mercury concentrations.

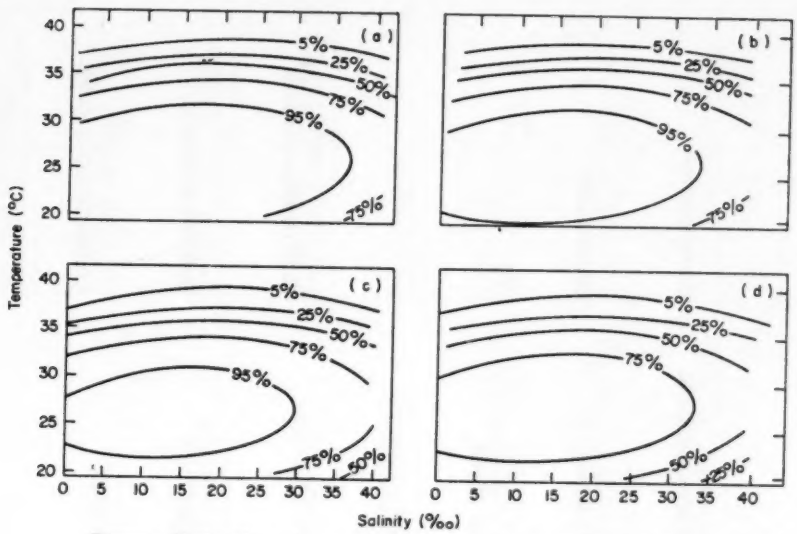

Figure 4. Estimation of percentage of survival of megalopae of *Rhithropanopeus harrisii* based on fitted response surface to observed survival under 64 combinations of salinity, temperature and mercury. (a) 0 parts 10^{-9} Hg^{2+}, (b) 5 parts 10^{-9} Hg^{2+}, (c) 10 parts 10^{-9} Hg^{2+} and (d) 20 parts 10^{-9} Hg^{2+}.

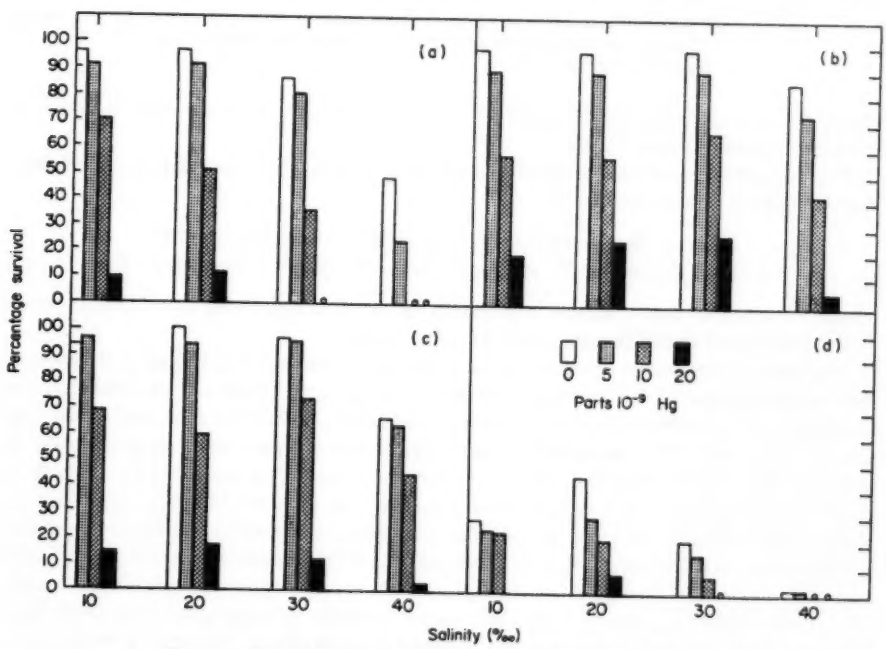

Figure 5. Percentage survival of all larval stages of *Rhithropanopeus harrisii* reared from hatch to first crab stage in 64 combinations of salinity, temperature and mercury. (a) 20 °C, (b) 25 °C, (c) 30 °C and (d) 35 °C.

Total larval survival from hatch to first crab

The combined influences of salinity, temperature and mercury on survival of *Rhithropanopeus harrisii* during total larval development from hatch through metamorphosis to first crab are shown in Figure 5. In general, the percentage of survivors to the first crab stage is inversely related to mercury levels from 5 to 20 parts 10^{-9} Hg^{2+}. Larvae developing at 25 and 30 °C appear more resistant to mercury toxicity than those reared at higher or lower temperatures. At the extreme temperatures of 20 and 35 °C, fewer larvae survived exposure to mercury when developing at salinities above 20‰.

Regression analysis of the survival data throughout total development produced a polynomial equation explaining 86% of the variation:

$$\hat{Y}_3 = -210{\cdot}02 - 6{\cdot}2901\mathrm{Hg} - 0{\cdot}0239\mathrm{Hg}^2 + 0{\cdot}4305S - 0{\cdot}0510S^2 + 24{\cdot}6902T - 0{\cdot}5184T^2 + 0{\cdot}0304\mathrm{Hg}\times S + 0{\cdot}1245\mathrm{Hg}\times T + 0{\cdot}0424S\times T, \quad (4)$$

where $\hat{Y}_3$ = the estimated arcsine $\sqrt{\text{percentage survival}}$ of all larval stages throughout development from hatch to first crab and the other terms follow the general model [equation (1)] described earlier.

Response surface curves of regression equation (4) produced for each experimental mercury concentration illustrate the reduced survival capacity of *Rhithropanopeus harrisii* in the presence of mercury during complete larval development under a broad range of salinities and temperatures (Figure 6). Absolute maximum survival values decrease from 99% in the absence of mercury to 31% in 20 parts 10^{-9} Hg^{2+}. It is estimated that 95% of the hatched larvae successfully complete metamorphosis in salinities from 0 to 33‰ and at temperatures from below 20 to slightly above 30 °C. Development in 10 parts 10^{-9} Hg^{2+} reduces the predicted envelope for 75% survival to more restricted salinity–temperature conditions,

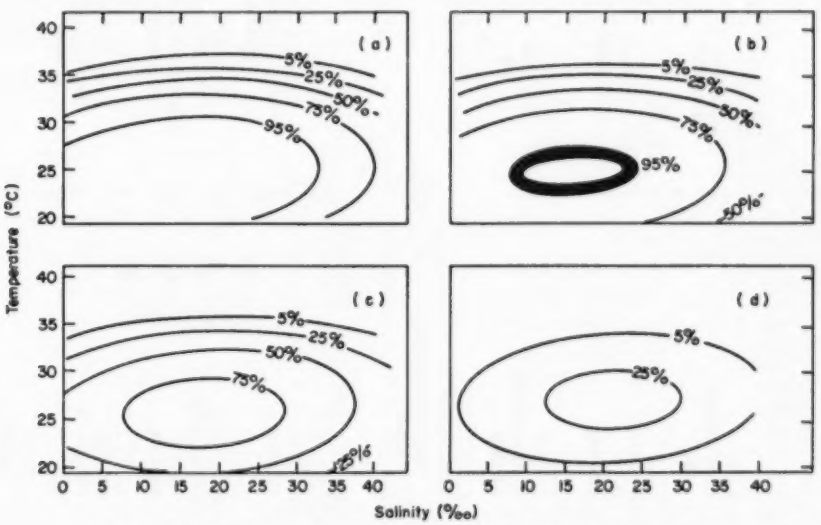

Figure 6. Estimation of percentage survival of all larval stages from hatch to first crab stage based on fitted response to observed survival under 64 combinations of salinity, temperature and mercury. (a) 0 parts 10^{-9} Hg^{2+}, (b) 5 parts 10^{-9} Hg^{2+}, (c) 10 parts 10^{-9} Hg^{2+} and (d) 20 parts 10^{-9} Hg^{2+}. The dark-banded area in (b) represents the range of combinations of temperature, salinity and mercury giving 95% survival.

while exposure to 20 parts 10^{-9} Hg^{2+} allows only 25% of the hatched larvae to complete development within the more limited physiochemical conditions of salinities ranging from 13 to 30‰ and temperatures between 25 and 30 °C.

In addition to the significant ($P = 0.02$) salinity–temperature interaction demonstrated by the slight declination of the horizontal axis of the survival contours (Figure 6), a significant ($P = 0.03$) mercury–salinity interaction affected the viability of developing larvae. Exposure to as little as 5 parts 10^{-9} Hg^{2+} reduced the salinity plasticity of developing larvae by restricting the 95% survival response from the broad salinity range of 0–33‰ to the more narrow range of 8–24‰. At higher mercury concentrations, larval resistance to mercury was directly related to an increase in salinity. The optimal salinity for the maximum survival response shifted with increasing mercury exposure toward higher salinities as reflected by the change in the center of the surface from a salinity of 14·4‰ in the absence of additional mercury to 16·1, 17·9 and 21·4‰ S upon exposure to 5, 10 and 20 parts 10^{-9} Hg^{2+}, respectively.

Similarly, the thermal plasticity of developing larvae was restricted by exposure to mercury, i.e. 95% of the larvae are predicted to metamorphose successfully to first crab at temperatures from below 20 to slightly above 30 °C, while the thermal range for similar success for those larvae developing in 5 parts 10^{-9} Hg^{2+} is restricted to a temperature range of 23 to 27 °C (Figure 6). Mercury interacts significantly ($P = 0.0001$) with temperature to

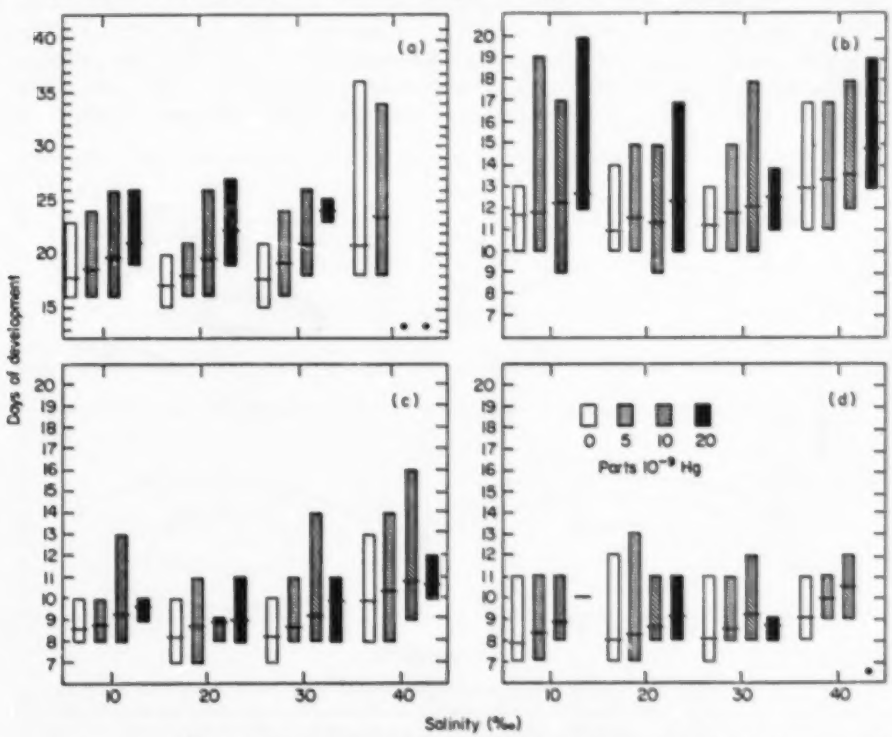

Figure 7. Minimum, maximum and mean days of zoeal development of *Rhithropanopeus harrisii* reared under 64 combinations of salinity, temperature and mercury. (a) 20 °C, (b) 25 °C, (c) 30 °C and (d) 35 °C.

reduce the thermal plasticity of larvae more at lower than at higher temperatures as demonstrated by the greater upwards shift of the lower thermal boundary upon exposure to higher mercury concentrations. Furthermore, this mercury–temperature interaction is reflected in the increased temperature level seen at the center of the response surface accompanying higher mercury concentrations, indicating a rise in optimal temperature for survival from 24·4 °C in 0 parts 10^{-9} Hg^{2+} to 27·1 °C in 20 parts 10^{-9} Hg^{2+}.

Zoeal developmental rates

The time for development of zoeae to the megalopal stage was prolonged by continuous exposure to increasing concentrations of mercury ranging from 5 to 20 parts 10^{-9} Hg^{2+} and these retardations in developmental rates were further dependent on salinity–temperature conditions (Figure 7).

Regression analysis of the duration data for the zoeal stages generated a second-order polynomial expression explaining 96% of the variation:

$$\hat{Y}_4 = 84{\cdot}80 + 0{\cdot}3499\mathrm{Hg} - 0{\cdot}0023\mathrm{Hg}^2 - 0{\cdot}0395S + 0{\cdot}0059S^2 - 4{\cdot}8987T + 0{\cdot}0801T^2 - 0{\cdot}0009\mathrm{Hg}\times S - 0{\cdot}0069\mathrm{Hg}\times T - 0{\cdot}0068S\times T, \quad (5)$$

where $\hat{Y}_4$ = the estimated days of development of all zoeal stages of *Rhithropanopeus harrisii* to megalopa and the other terms follow the general model [equation (1)] described earlier.

The response surface curves developed from equation (5) display the combined effects of salinity, temperature and mercury on the developmental rates of zoeae of *Rhithropanopeus harrisii* (Figure 8). The slight rotation of the horizontal axis demonstrates the highly significant ($P = 0{\cdot}0001$) interaction between salinity and temperature on developmental

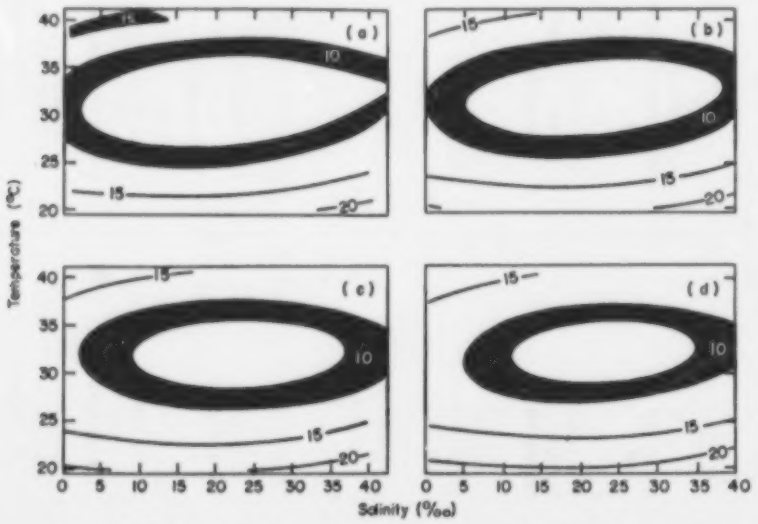

Figure 8. Estimation of days of development of all zoeal stages of *Rhithropanopeus harrisii* based on fitted response surface to observed duration under 64 combinations of salinity, temperature and mercury. (a) 0 parts 10^{-9} Hg^{2+}, (b) 5 parts 10^{-9} Hg^{2+}, (c) 10 parts 10^{-9} Hg^{2+} and (d) 20 parts 10^{-9} Hg^{2+}. The dark-banded areas represent the range of combinations of temperature, salinity and mercury for the given duration.

rates of zoeae, irrespective of mercury level. This rotation suggests that maximum developmental rates of zoeae were attained when an increase in temperature is coupled with a slight increase in salinity. Developmental rates of zoeae were reduced in the presence of mercury from an absolute minimum developmental period of 7·3 days in the absence of additional mercury to 8·6 days in 20 parts 10^{-9} Hg^{2+}. In addition, exposure to increasing concentrations of mercury reduced the thermal range for the minimum developmental period of 10 days from 25–37·5 to 28–37 °C, indicating a significant ($P = 0{\cdot}0004$) mercury–temperature interaction again affecting the lower end of the thermal range more than the upper.

Megalopal developmental rates

Figure 9 illustrates the interactions of mercury, salinity and temperature on the developmental duration of megalopa to first crab. While, in general, the developmental rates of megalopae appear less affected by mercury exposure than the zoeae, their susceptibility remained dependent on salinity and temperature conditions during exposure.

The regression equation explaining 90% of the variation of the megalopae developmental data is:

$$\hat{Y}_s = 47{\cdot}52 + 0{\cdot}1977\mathrm{Hg} - 0{\cdot}0014\mathrm{Hg}^2 - 0{\cdot}2283S + 0{\cdot}0031S^2 - 2{\cdot}6945T + 0{\cdot}0415T^2 + 0{\cdot}0014\mathrm{Hg}\times S - 0{\cdot}0049\mathrm{Hg}\times T + 0{\cdot}0042S\times T, \quad (6)$$

where $\hat{Y}_s$ = the estimated days of development from megalop to first crab and other terms follow the general model [equation (1)] described earlier.

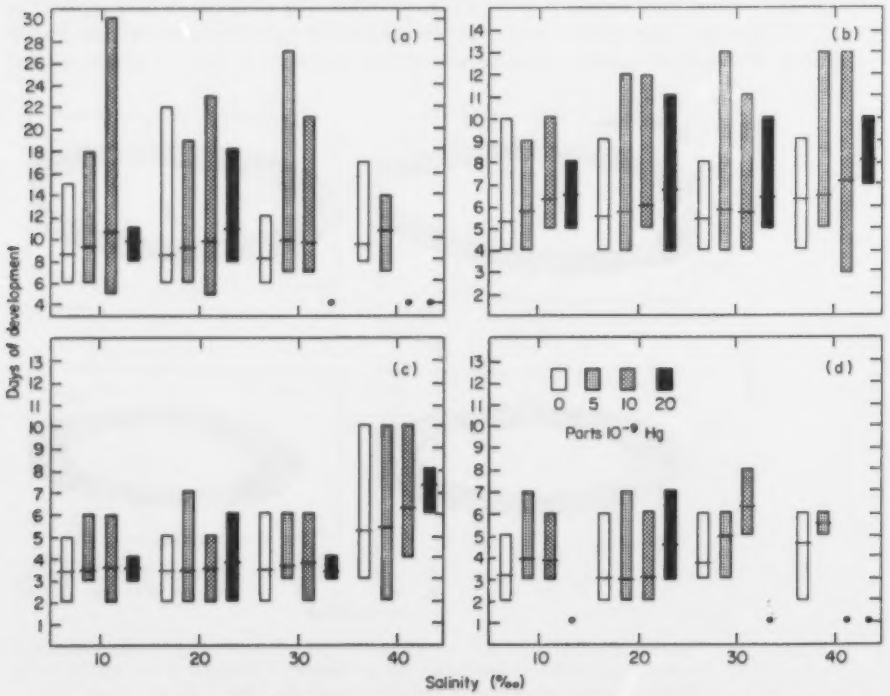

Figure 9. Minimum, maximum and mean days of megalopal development of *Rhithropanopeus harrisii* reared under 64 combinations of salinity, temperature and mercury. (a) 20 °C, (b) 25 °C, (c) 30 °C and (d) 35 °C.

The response surface curves developed from equation (6) show the significant ($P = 0{\cdot}03$) interaction between salinity and temperature on developmental rates of megalopae (Figure 10). However, for megalopae the horizontal axis rotates in the opposite direction from that for zoeae, indicating that megalopae tend to develop more rapidly when increased temperature is coupled with a decrease in salinity. As with zoeae, maximum developmental rates of megalopae exist within a specific thermal range (25–37·5 °C) which becomes restricted, particularly at the lower end, with increasing mercury concentrations (at 20 parts 10^{-9} Hg^{2+}, 28·5–37·5 °C). In addition to this significant ($P = 0{\cdot}05$) mercury–temperature interaction, megalopal development, unlike that of zoeae, was markedly ($P = 0{\cdot}07$) affected by a mercury–salinity interaction. With exposure to increasing concentrations of mercury, the salinity range for the minimal developmental period for megalopae (4 days), which occurred at salinities from 0 to 38‰ in the absence of additional mercury, became more restricted, i.e. 0–35 in 5 parts 10^{-9} Hg^{2+}, 0–31 in 10 parts 10^{-9} Hg^{2+}, and 0–27‰ S in 20 parts 10^{-9} Hg^{2+} (Figure 10). Similarly, the optimal salinity for the maximum developmental rate of megalopae (the center of the response surface) decreased from 15·4‰ S in the absence of additional mercury to 14·0, 12·6 and 9·8‰ S upon exposure to 5, 10 and 20 parts 10^{-9} Hg^{2+}, respectively.

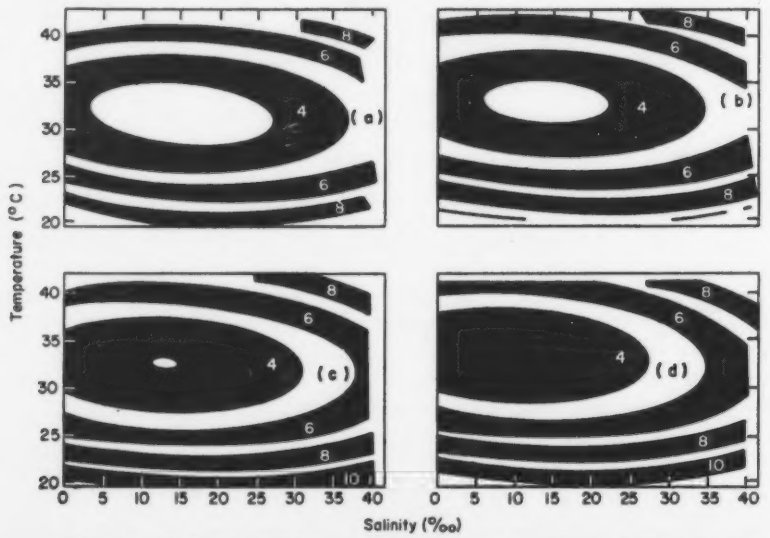

Figure 10. Estimation of days of development of megalopae of *Rhithropanopeus harrisii* based on fitted response surface to observed duration under 64 combinations of salinity, temperature and mercury. (a) 0 parts 10^{-9} Hg^{2+}, (b) 5 parts 10^{-9} Hg^{2+}, (c) 10 parts 10^{-9} Hg^{2+} and (d) 20 parts 10^{-9} Hg^{2+}. The dark-banded areas represent the range of combinations of temperature, salinity and mercury for the given duration.

Developmental rates from hatch to first crab

As with development through the four zoeal stages and the megalopal stage, the duration of total development from hatch to first crab was jointly retarded by various conditions of salinity, temperature and mercury (Figure 11). As might be expected, lower temperatures decreased the rate of total development through metamorphosis. Increasingly higher concentrations of mercury retarded metamorphosis, irrespective of temperature and salinity.

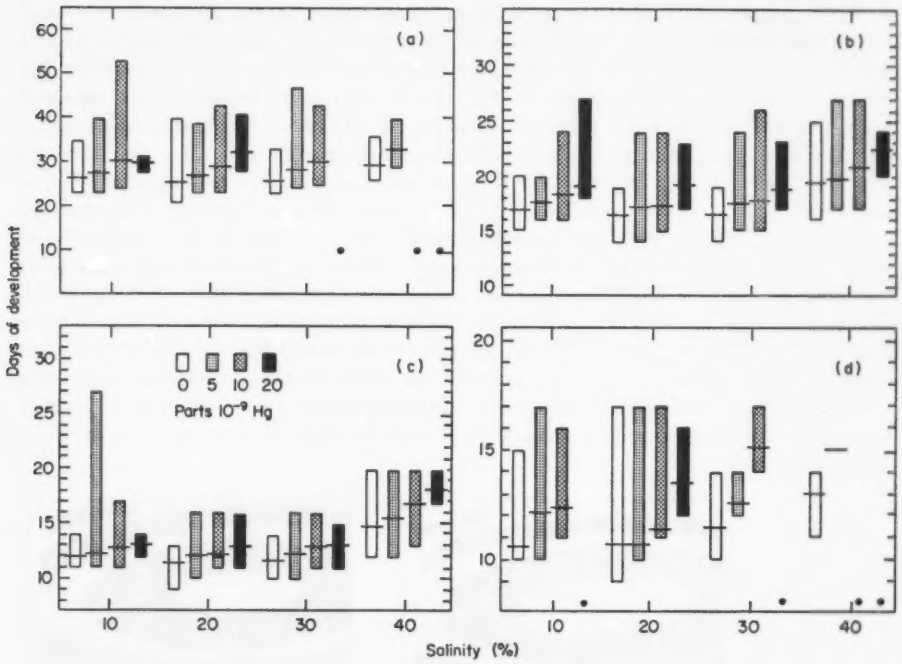

Figure 11. Minimum, maximum and means days of total larval development of *Rhithropanopeus harrisii* reared from hatch to crab under 64 combinations of salinity, temperature and mercury. (a) 20 °C, (b) 25 °C, (c) 30 °C and (d) 35 °C.

With few exceptions and at all temperatures, salinities above 20‰ in conjunction with elevated mercury concentrations prolonged the time required for successful development to juvenile crabs.

Multiple linear regression analysis of the total developmental duration data produced a polynomial equation explaining 96% of the variation:

$$\hat{Y}_6 = 128{\cdot}74 + 0{\cdot}4764\mathrm{Hg} - 0{\cdot}0044\mathrm{Hg}^2 - 0{\cdot}3209S + 0{\cdot}0080S^2 - 7{\cdot}2712T + 0{\cdot}1147T^2 + 0{\cdot}0013\mathrm{Hg}\times S - 0{\cdot}0095\mathrm{Hg}\times T + 0{\cdot}0004S\times T, \quad (7)$$

where $\hat{Y}_6$ = the estimation of days required from hatching to first crab for *Rhithropanopeus harrisii* and the other terms follow the general model [equation (1)] described earlier.

Response surface curves developed from equation (7) demonstrate the combined effects of salinity, temperature, and mercury on the developmental rates of *Rhithropanopeus harrisii* from hatch to completion of metamorphosis to first crab (Figure 12). The capacity for total development in 10 days at temperatures of 31–32 °C and in salinities from 18–22‰ is obliterated upon exposure to 5 parts 10^{-9} Hg^{2+}. The absolute minimum developmental period is increased from 9·5 days in the absence of additional mercury to 12·7 days in 20 parts 10^{-9} Hg^{2+}.

A significant ($P = 0{\cdot}015$) mercury–temperature interaction exists whereby developmental rates of larvae are more sensitive to retardation by mercury exposure at lower temperatures than for those at higher temperatures. Estimated duration contours at the lower end of the

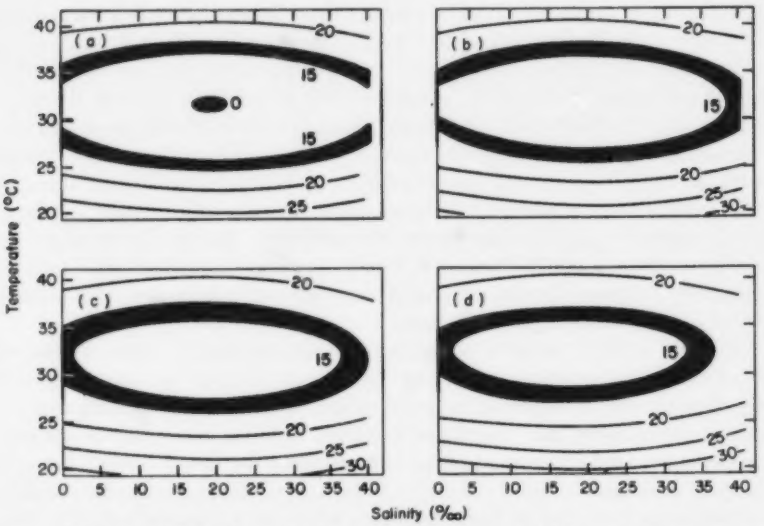

Figure 12. Estimation of days required from hatching to first crab of *Rhithropanopeus harrisii* based on fitted response surface to observed duration under 64 combinations as salinity, temperature and mercury. (a) 0 parts 10^{-9} Hg^{2+}, (b) 5 parts 10^{-9} Hg^{2+}, (c) 10 parts 10^{-9} Hg^{2+} and (d) 20 parts 10^{-9} Hg^{2+}. The dark-banded areas represent the range of combinations of temperature, salinity and mercury for the given duration.

temperature scale are shifted upward with increasing concentrations of mercury. For example, the lower limit of the thermal range for total development in 15 days is restricted from 25 °C in the control larvae to 26 °C in 5 parts 10^{-9} Hg^{2+}, 27 °C in 10 parts 10^{-9} Hg^{2+} to 27·5 °C in 20 parts 10^{-9} Hg^{2+}.

Discussion

Survival

Comparison of the response surface curves of larval survival under a broad range of salinities and temperatures of *Rhithropanopeus harrisii* from a North Carolina population (Costlow *et al.*, 1966) and from a Florida population, as reported in this study, indicate a greater degree of salinity plasticity among larvae from the Florida population. These differences in estimated responses might reflect dissimilarities in experimental design [Costlow *et al.* (1966) employed a larger number of experimental salinity conditions], phenotypic differences reflecting differential previous salinity experiences of the deposited eggs (Kinne, 1962; Vernberg & Vernberg, 1972), or actual genetic differences between the two populations, resulting from isolation between the two populations experiencing different salinity conditions as determined by variations in the hydrography of the two estuarine habitats. Aside from these apparent differences in the salinity resistance, thermal resistances of larvae from both populations are quite similar.

Observations from the present study demonstrate that mercury concentrations from 5 to 20 parts 10^{-9} Hg^{2+} reduce the survival capacity for developing larvae of *Rhithropanopeus harrisii* (as indicated by changes in absolute magnitude of the survival response surface).

Comparison of responses of the two larval forms suggest that megalopae are more resistant to mercury toxicity than earlier zoeal stages. These results agree with those of another study (McKenney & Costlow, unpublished manuscript, 1978), in which discreet zoeal and megalopal stages were exposed to mercury for similar time periods, thereby eliminating confusion of direct stress effect with selection. Other studies have shown differential sensitivity between developmental stages of several brachyuran species to various toxicants with the earlier stages being less resistant in some cases (Buchanan *et al.*, 1970; Epifanio, 1971; Katz, 1973; Bookhout & Costlow, 1975; Rosenberg & Costlow, 1976; Christiansen *et al.*, 1977*a*, *b*, 1978) and later stages more sensitive in others (Bookhout & Costlow, 1970; Bookhout *et al.*, 1972; DeCoursey & Vernberg, 1972; Costlow, 1977). Thus, the degree of sensitivity between discreet developmental stages of brachyurans to a toxicant may vary with both species and type of toxicant.

Developing larvae were more resistant to mercury toxicity under optimal conditions of temperature and salinity (25 °C and 15–20‰ *S*) (Figure 6), while mercury interacted significantly with both temperature and salinity to alter the survival capacity of developing larvae. Both zoeae and megalopae exhibited reduced resistance to mercury at lower temperatures and, in turn, optimal temperatures for larval survival were higher with increasing concentrations of mercury. Concurrently, tolerance to mercury during total larval development decreased at lower salinities, while the optimal salinity for complete larval development shifted upward with increasing mercury concentrations. Similar to these results, the mortality of larval *Uca pugilator* exposed to mercury increased at suboptimal temperatures and salinities (Vernberg *et al.*, 1973*b*). The toxicity to mercury to three species of marine and brackish-water isopods dramatically increased in both lower salinities and higher temperatures than considered optimal for survival (Jones, 1973).

The response surface curves of survival when interpreted in terms described by Alderdice (1972) suggest that exposure to mercury accompanies a decreased salinity and temperature plasticity of the normally euryhalinic and eurythermal larvae of *Rhithropanopeus harrisii*. In the presence of as little as 5 parts 10^{-9} Hg^{2+}, the predicted salinity–temperature range for 95% of the larvae to successfully complete development decreased from 0–30‰ *S* and 20–30 °C to 8–24‰ *S* and 23–27 °C (Figure 6). While this phenomena exists for both zoeal stages and megalops, exposure to mercury is associated with a greater reduction in salinity–temperature plasticity of zoeae than of megalopae.

Larvae of *Rhithropanopeus harrisii* remain isosmotic with seawater of 30–40‰ *S*, while at salinities lower than 30‰ *S* larvae actively hyperregulate (Kalber & Costlow, 1966). Reduced resistance of larvae to mercury at lower salinities may reflect disruptions of their normal osmoregulatory processes as has been demonstrated in the adult crabs of *Carcinus maenus* and *Cancer irroratus* upon exposure to cadmium and copper (Thurberg *et al.*, 1973). Similarly, a number of heavy metals, including mercury, significantly altered the osmoregulatory ability of the estuarine isopod *Jaera albifrons* in low salinities (Jones, 1975). Exposure to sublethal concentrations of methylmercury reduced the haemolymph sodium concentrations of the amphipod *Gammarus duebeni* in low salinities, as well as depressing the maximal and steady-state sodium transport rates (Inman & Lockwood, 1977).

Developmental rates

Results from this study are in agreement with those from other studies which indicate that both salinity and temperature may significantly modify the developmental rates of brachyuran larvae (Costlow *et al.*, 1960, 1962, 1966; Ong & Costlow, 1970; Lucas, 1972). Of these two abiotic environmental factors, temperature plays the dominant role with lower temperature

reducing developmental rates. On the other hand, extreme salinities might also retard developmental rates, but not to the extent seen with lower temperatures.

Developmental rates for the zoeae and megalopae of *Rhithropanopeus harrisii* from a Florida population as reported in this study are similar to those reported for the same species from a North Carolina population (Costlow *et al.*, 1966) when comparing the rates of each larval form separately. However, response surface curves estimating time for total development for the Florida larvae (Figure 12) produced a 10-day contour within very restricted salinity–temperature conditions (18–22‰ *S* and 31–32 °C), while this minimum contour for North Carolina larvae was one of 15 days. These apparent differences between larvae from the two populations might be misleading, in that, choice of contour level is a product of the computer program and, further, 30 °C was the highest experimental temperature used to produce the contours for the North Carolina larvae, while experimental larvae in this study experienced 35 °C. In addition, Florida larvae were predicted to have a minimal developmental time period at a more expanded upper salinity range than North Carolina larvae (particularly noticeable for megalopae in Figure 10, this study, and figure 19, Costlow *et al.*, 1966). As with the discrepancies between the two populations in survival capacities, these differences might reflect genotypic or phenotypic dissimilarities between populations or non-comparable experimental procedures.

Increasing concentrations of mercury from 5 to 20 parts 10^{-9} Hg^{2+} progressively retarded the developmental rates of zoeae to a greater degree than megalopae (Figures 7, 9). This sublethal effect of heavy metal exposure has been reported for other decapod larvae. Acute exposure to sublethal concentrations of mercury extended the duration for total larval development of *Palaemonetes vulgaris* (Shealy & Sandifer, 1975). Developmental rates of the caridean shrimp *Palaemonetes pugio* were retarded by continual exposure to a sublethal concentration of zinc throughout total larval development (McKenney & Neff, 1979). Noting that reductions in developmental rates accompany both sublethal exposure to various toxic substances and stress salinities, Epifanio (1971) suggests that this retardation in the molting process may be a generalized response of brachyuran larvae to any environmental stress.

Mercury interacted with temperature and with salinity to alter the developmental rates of larval *Rhithropanopeus harrisii*. Developmental rates of megalopae were more reduced by exposure to mercury at higher salinities (Figure 10). In accordance, the salinity for minimal developmental time for megalopae decreased from 15 to 10‰ *S* upon exposure to 20 parts 10^{-9} Hg^{2+}. For both zoeae and megalopae, a significant mercury–temperature interaction existed by which the time for development of both larval forms was prolonged more by mercury at lower than at higher temperatures (Figures 8, 10). Similarly, a significant zinc–temperature interaction reduced developmental rates of *Palaemonetes pugio* larvae more at suboptimal temperatures (McKenney & Neff, 1979).

Ecological implications

Since the actual concentrations of mercury experienced by the larvae in this study (Table 1) approach concentrations released into and reported for heavily polluted estuaries (Klein & Goldberg, 1970; Friberg & Vostal, 1972; Bryan, 1976), a number of ecological implications may be inferred from the results. Those mercury concentrations responsible for diminished larval survival further reduce the naturally low survival of pelagic larvae (Thorson, 1950) and, subject to the influence of density dependent mortality, may adversely alter recruitment into the parental benthic populations (Mileikovsky, 1971; Sandifer, 1973*b*, 1975).

To exist under estuarine conditions, organisms must be able to tolerate extreme ranges and intensity variations of both temperature and salinity (Kinne, 1964, 1970, 1971; Vernberg

& Vernberg, 1972). In the presence of low concentrations of mercury, a reduction in the temperature–salinity plasticity of the normally eurythermal and euryhalinic larvae of *Rhithropanopeus harrisii* might alter the success of this species within the estuary. Mercury exposure, furthermore, disrupts the physiological capabilities of the larvae that would allow them, as postulated by Costlow *et al.* (1966), to be transported from one estuary to another by offshore currents. Thereby, a lessened potential exists for larvae to colonize new habitats for the species or to re-establish damaged neighbouring adult populations (Mileikovsky, 1970, 1971).

Rhithropanopeus harrisii displays a broad latitudinal distribution, ranging from the Miramichi Estuary, Canada (Bousfield, 1955) to Lake Maracaibo, Venezuela (Rodriguez, 1963). Larvae of *R. harrisii* appear in the plankton from May until September in the Newport River Estuary in North Carolina (Pinschmidt, 1963), while ovigerous females can be found throughout the year in the Indian River coastal zone in Florida (R. Laughlin, personal communication). Enhanced toxicity of mercury at lower temperatures may limit distribution of the species in cooler waters and shorten successful reproductive periods at all latitudes, eliminating viable larvae during cooler months of the year.

Pinschmidt (1963) collected zoeae of *Rhithropanopeus harrisii* in the plankton between salinities of 0 and 33‰ with more larvae present in bottom samples and a peak abundance found well up the estuary in 16‰ *S*. Similarly, *R. harrisii* larvae were found principally in the bottom waters of the estuaries of the York River and lower Chesapeake Bay and the proportion on the bottom increased with the stage of development (Sandifer, 1973*b*). These field observations support the hypothesis that this species may possess behavioural mechanisms of vertical migration with changing tidal cycles, which act to retain the larvae within the estuary (Bousfield, 1955; Sandifer, 1975). The reduced salinity plasticity of larvae developing in the presence of low concentrations of mercury and the enhanced toxicity of mercury at lower salinities may alter both horizontal and vertical distribution of the larvae within the water column and, consequently, limit successful retention within the estuary. In addition, this reduced survival capacity of larvae in low salinity waters may increase interspecific competition from many parasites and predators (Thorson, 1957).

The sublethal responses to mercury of a 3- to 4-day prolongation of the larval period permits a continuance of this pelagic phase in the life cycle of the species. The result of this prolonged pelagic period may include increased predation on the susceptible larvae, hence reduced recruitment into the adult population, and a lessened probability for retention of the larvae within the estuary.

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